USER GUIDE

VectaPlex™ Antibody Removal Kit

vector LABORATORIES

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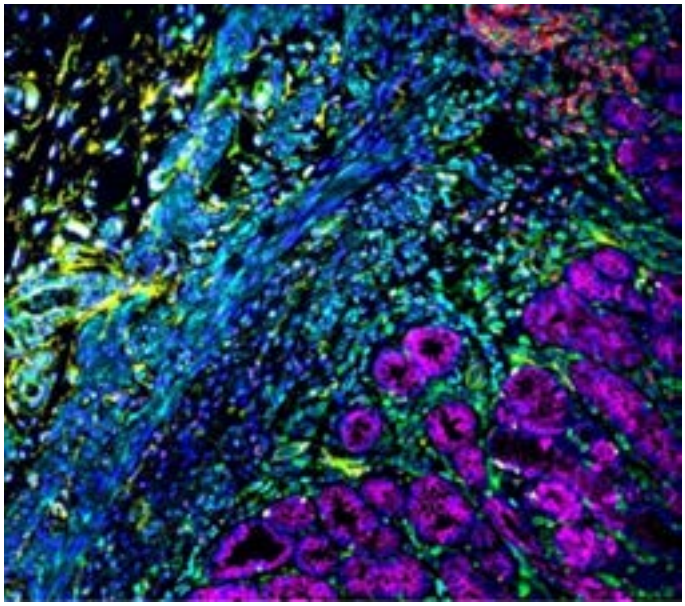
Cat. No. VRK-1000

Storage Ambient (15–25° C)

Description This ready-to-use kit is designed to remove antibodies and other non-covalently bound detection reagents from formalin-fixed paraffin-embedded (FFPE) tissue sections in immunofluorescence or tyramide signal amplification staining workflows.

Components

Product Name	Volume
VectaPlex™ Antibody Removal Reagent A	25 ml
VectaPlex™ Antibody Removal Reagent B	25 ml



Stomach section stained with 6 mouse primary antibodies (CD20 (Red), CD34 (Yellow), DES (Cyan), AE1/AE3 (Magenta), CD3 (Grey), VIM (Green)) detected with DyLight™ 488 horse anti-mouse IgG and mounted with VECTASHIELD® PLUS with DAPI. This composite image was generated from 6 rounds of immunofluorescent staining using the VectaPlex™ Antibody Removal Kit.

Instructions for Use

Option 1: Immunofluorescence Workflow

Preparation for Procedure

Complete standard immunofluorescence staining procedure. Mount with a non-hardening (non-setting) mounting medium and image slide.

Procedure

1. Remove coverslip from tissue section and wash thoroughly with PBS pH 7.4 to remove residual mounting medium.
2. Add sufficient VectaPlex™ Reagent A to cover tissue section completely (typically ~ 150–200 µL) and incubate for 15 minutes at room temperature.
3. Wash slides briefly with PBS.
4. Add sufficient VectaPlex™ Reagent B to cover tissue section completely (typically ~ 150–200 µL) and incubate for 15 minutes at room temperature.
5. Wash slides with PBS for 5 minutes.
6. The tissue section is now ready to begin another immunofluorescence staining procedure with a protein blocking step followed by the next round of antibody staining, mounting, and imaging.
7. Repeat until the desired number of antigens have been detected.

Option 2: Tyramide Signal Amplification Workflow

Preparation for Procedure

Complete standard tyramide signal amplification staining procedure.

Procedure

1. Add sufficient VectaPlex™ Reagent A to cover tissue section completely (typically ~ 150–200 µL) and incubate for 15 minutes at room temperature.
2. Wash slides briefly with PBS.
3. Add sufficient VectaPlex™ Reagent B to cover tissue section completely (typically ~ 150–200 µL) and incubate for 15 minutes at room temperature.
4. Wash slides with PBS for 5 minutes.
5. Tissue sections are now ready to begin another fluorescent tyramide signal amplification staining procedure with a protein blocking step followed by the next round of antibody staining using a differently colored dye.
6. Repeat until the desired number of antigens have been detected.
7. Once staining is completed, mount tissue sections and image.

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Notes:

1. **Mixing VectaPlex reagents with bleach may result in the formation of a hazardous gas.** For this reason, do not mix reagents or waste solutions from this process with bleach, and use reagents in a well-ventilated area using appropriate laboratory personal protective equipment (PPE).
2. When performing the immunofluorescence workflow, images from successive staining cycles can be registered and overlaid using free software such as Fiji (https://fiji.sc/).
3. When performing the immunofluorescence workflow, use of a mounting medium containing DAPI during each staining cycle facilitates image alignment.
4. This product has been developed for use across a wide range of FFPE sample and staining conditions. Up to six rounds of successive staining and antibody removal have been demonstrated. However, performance may vary depending on the sample or experimental design used. For this reason, control experiments using single-plex staining with and without VectaPlex usage are recommended.

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